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OFFICE OF TECHNOLOGY LICENSING
INVENTION AND TECHNOLOGY DISCLOSURE
(see instructions on reverse side)

CASE NUMBER
B 92-011

LOG DATE

COMPLETE ITEMS 1-6 - USE ADDITIONAL SHEETS AS NECESSARY

1. TITLE OF INVENTION

Chemical Flow and Reactor Microinstrumentation

2. INVENTOR(S)

INVENTOR(S)	TITLE	CAMPUS UNIT OR MAILING ADDRESS
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3. CONTRACT OR GRANT NO.(S)

CONTRACT OR GRANT NO.(S)	SPONSOR(S)	PRINCIPAL INVESTIGATOR
N/A	BSAC	

4. EVENTS

EVENTS	DATE	REFERENCES & COMMENTS
A. Initial idea		Telephone conversation between inventors
B. First description of complete invention, oral or written (conception)*		Previous Disclosures
C. First successful demonstration, if any (first actual reduction to practice)*		Notebook - M.A.N
D. First publication containing full description of invention (establishment of publication bar)*	NA	
E. External oral disclosures	N/A	

5. BRIEF ABSTRACT OF INVENTION - ATTACH DETAILED DESCRIPTION

Application of Micro-structures to microreactions specific embodiment is application to PCR (See Attached details)

KEYWORDS (OTL USE ONLY)

6. INVENTION SUBMITTED BY:

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Katalin Voros
Witness' Signature** Date

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** Please have PI sign if PI is not an inventor.

Invention Disclosure

Chemical Flow and Reactor Microinstrumentation

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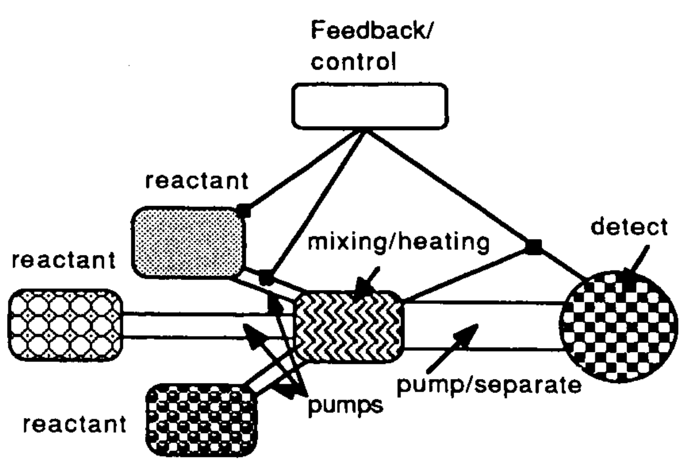
Introduction

Microfabrication technologies allow the design and manufacture of microstructures for performing small volume chemical reactions, manipulations, and detection. This disclosure describes the application of multiple microfabricated devices and their integration into micro-sized instruments for chemical reaction control, product and reactant manipulations, and detection of participating reactants and resultant products.

Micro-reactors consisting of multiple inlet and output pumps and valves, heaters, electromechanical devices for process enhancement, and detection capabilities can be made through microprocess technology. Integration of a few to many of these type of devices into a single instrument will provide a complete instrument for chemical processing and detection. Examples of such integrated microinstruments include but are not limited to biochemical, inorganic and organic chemical reactors, biomedical and environmental diagnostic and therapeutic devices, and biotechnological processing and detection microinstruments. An example embodiment of the described microinstrument will be described for the processing and detection of DNA-based biotechnology.

Invention Overview

The invention herein describes a microinstrument based on microfabricated parts that perform reactant and product manipulations and detection. Included are heaters, pumps, detection methodology, surface treatments, and reactant and/or product separations. Examples of such microfabricated components are 1) micro-heaters consisting of polysilicon or other appropriate material patterned onto and made an integral part of the microstructure, 2) micro-pumps made of Lamb-wave devices (U.S. Patent No. 5,006,749, R.M. White, 1991) or electrokinetic pumps, or other microfabricated pump structure, 3) micro-detection methodology such as fluorescence-based optical fiber spectroscopy with microfabricated light sources and detectors (e.g., LEDs or diode lasers and detectors) or Lamb-wave sensors (Patent pending, R.M. White et al.), or other appropriate detection methodology, 4) micro-device surface treatments for reaction enhancement, product separation, and detection can be based on numerous well-known procedures such as silanol-based derivatizations or other appropriate treatments. 5) separations can be based on micro-electrophoresis either in a capillary or within a gel, or can be based on other appropriate methodologies. Integrated control and feedback, and results interpretation can be contained on the same microinstrument.



Example of an integrated micro-reactor and instrument

Figure 1

Specific Example of the Embodiment: Microinstrumentation-Based Polymerase Chain Reaction (PCR) and Diagnostics

Background

The polymerase chain reaction (PCR) is a method by which a single molecule of DNA (or RNA) of an organism can be selectively amplified several millionfold within a few hours. This well-established procedure is based on the repetition of heating (denaturing) and cooling (annealing) cycles in the presence of the original DNA molecule, specific DNA primers, dNTPS, and DNA polymerase enzymes. Each cycle produces a doubling of the target DNA segment, leading to an exponential accumulation of the target segment. The generalized procedure involves: 1) processing of the sample to release target DNA molecules into a crude extract, 2) addition of an aqueous solution containing enzymes, buffers, deoxyribonucleotide triphosphates (dNTPS), and two oligonucleotide primers, 3) thermal cycling of the reaction mixture at two or three temperatures (i.e., 90-96, 72, and 37-55 °C) for typically 20 to 40 cycles, and 4) detection of amplified DNA . Intermediate steps are introduced in some assays to incorporate signal-producing and/or surface-binding primers, and to purify the reaction products (e.g., electrophoresis or chromatography. Reaction volumes and times are typically on the order of tens of μLs and one to two hours, respectively. PCR-based technology has been applied to a variety of analyses, including environmental and industrial contaminant identification, medical and forensics diagnostics, and biological research.

Invention Concept

The invention disclosure herein concerns the application of microinstrumentation to PCR. The small analytical and reaction volumes of PCR make it an ideal diagnostic technique for implementation on micro-devices. The potential increase in amplification and reaction speed afforded by miniaturization will contribute to increased parallelism and a decrease in analysis times. Such a system could contain reservoirs of reagents; agitation, vortexing, and mixing devices to process the analytes; pumps to carry reagents to mixing chambers; heaters to perform the denaturing and annealing cycles; optical and/or electromechanical/chemical sensors to discriminate the reagents and products of the reaction; and separation devices to purify reactants and products. Feedback control could also be incorporated directly into the system.

The use of micro-manipulation and treatment of PCR-based technologies in microdevices can allow highly automated DNA technologies due to its ability to perform many reactions and manipulations with precise control

of temperature, evaporation, small-volume reagent delivery, product separation and isolation, and *in situ* reaction and product detection in parallel. Such highly automated and in parallel technologies will go far to expedite the use of DNA-based technologies for biomedical (e.g., the Human Genome Project), environmental (e.g., contaminant identification) and industrial (e.g., biotechnology) applications.

Figure 2 attached

PCR reaction could be performed in an isolated, surface-treated, sterile, temperature-controlled chamber as indicated in Figure 2. Polysilicon heaters for providing the denaturing and annealing temperatures can be incorporated into the top and/or bottom of the device chamber. Mixing of the fluid in the chamber can be provided by Lamb-wave membranes on either or both sides. In this way, the fluid is physically cycled between the two temperatures (and through the intermediate temperature for extension as well).

PCR in a microdevice can be just one step in a series of manipulations and conditions leading to the diagnostic detection of a variety of target species and the use of PCR products in genetic engineering. Amplification via PCR, as manipulated and controlled with micro-systems, yields products themselves that are subject to enhancement and detection with such devices. The control of reactions and conditions, and the physical manipulation of pre-PCR and post-PCR products and reagents can also be performed. The physical and chemical control via microdevices of cells and reagents prior to and after the production of PCR products will expand the number of the potential applications of DNA-based processes and analyses.

Pre-PCR manipulation of target cells or microorganisms can be accomplished with microdevices similar to that indicated in Figure 2. One example of many possible treatments is physical and/or chemical inducement to cell lysis. The use of the properties of the device (e.g. ultrasonic waves, surface states, and coating materials) can be used to manipulate cells and cell-contents, as can chemical treatments created by stirring and or mixing reagents from other areas and volumes on the integrated microinstrument. Sonication on a macro-scale in conjunction with microparticles, for example, has been used to facilitate the extraction of DNA from fixed cells (Heller et al.). Strategies similar to this, relying on the inherent properties of a microdevice, can be used to prepare intact cells, microorganisms, tissues, and other analytical samples for PCR and subsequent techniques. Two examples of many possibilities of this are the use of ultrasonic waves to disrupt and expose cell components through lysis, and to unravel large or long chain molecules such as DNA and proteins via disruption of secondary structure. Physical and chemical treatments such as these can also be incorporated into the PCR and post-

PCR phases of micro-device-based treatments to augment the reactions *in situ*.

Potential post-PCR treatments are numerous, but just the principle with a few examples will be described herein. In general, PCR on a microdevice is an integral part of the potential application of a device to further biotechnological manipulations and analyses. In other words, once PCR has been performed on a microdevice, post-PCR manipulations can lead to a myriad of possible microdevice-based DNA analyses and treatments. A few examples of such analyses are: large- and small-scale DNA sequencing of target species, cell-typing, analysis of PCR products with DNA probes, DNA fingerprinting, DNA cloning, physical mapping of genes and the maintainence of DNA libraries. Such analyses can lead to the use of DNA as vectors to produce cells or other biological entities to make desired products such as proteins or other therapies, or it can be used to produce DNA for use in therapies or biotechnological processes.

Analysis of PCR products, sequences of target DNA, or synthetic analogues in microdevices can be accomplished with the manipulative capabilities of microfabricated electrical and mechanical machines. For example, multi-dimentional arrays of pre-determined DNA sequences (probes) can be used to detect or verify PCR products, and their subsequent analyses can be accomplished with microdevices. Immobilization of biochemical molecules onto electronic devices and subsequent detection has been accomplished (Ref. Pharmacia, Molecular Devices, Naval Research Laboratory). The integration of similar surfaces within microdevice-based integrated systems is one example of the present application. Direct DNA sequencing of PCR products (single or double-stranded), can be accomplished with the use of unique temperature, enzymatic, strand separation schemes, and detection methodologies; all of which can be incorporated into a microdevice. Detection windows, reflective and absorptive surfaces, optic sources and other optical components can be fabricated and integrated onto a microdevice instrument, providing optical detection capability. Signal output and data analyses can be accomplished with on-board electronics.

PCR products may also be manipulated in order to be incorporated into genetic engineering vectors such as plasmids. Such vectors may subsequently be incorporated into target cells for the production of desired compounds. The target cells or moieties and reagents can be stored in reservoirs on the integrated device, released for exposure to the vectors while the physical/chemical conditions are established with the device. One other potential application would be the *in situ (in vitro* or *in vivo)* release of PCR products for direct genetic therapy or manipulations.

Many or all of the devices described above can be made from microfabrication technology and could process micro- to picoliter volumes. By the selection and integration of appropriate microfabricated devices, a precise and reliable reaction and analysis instrument for PCR-

based diagnostics could be devised. A schematic diagram of one such possible system is presented in Figure 1. Many of these microinstruments could be manufactured on a wafer and could run in parallel, allowing the processing and analysis of several target agents and controls. Target DNA detection methodology could include either an optical, electromechanical, electrochemical, or a combination sensing device. Detection signals could be processed and stored with microelectronic devices. Post-PCR tasks that can be performed by microdevices include but are not limited to examples such as incorporation of genetic vectors, DNA recombination, cell cloning, genetic therapy, and treatment and testing of biotechnological processes. As well, the incorporation of detection methodology, such as, but not limited to optical methodology, is included as it pertains to the the detection of pre-, concurrent, and post-PCR-processes on an integrated PCR-based microinstrument.

Summary

This disclosure describes an integrated microdevice-based instrument to perform chemical reactions and detection on a microscale. Monolithic microfabrication technology has advanced to the point where a variety of of micro-scale components can be made that have electrical, mechanical, optical, chemical, and thermal capabilities. For example, devices have been fabricated that can pump, heat, and mix microliter quantities of solids and liquids. As well, micro-scale optical and electromechanical/chemical physical and chemical sensors have been developed such as fiber optic probes and Lamb-wave sensors. The integration of these devices into systems allows the development of reactor-based analytical instruments on a micro-scale. The advantages of such integrated devices include the ability to manufacture them in batch quantities with high precision, yet low cost. Their inherent small size also provides significant advantage in that they would be able to perform very rapid, highly automated, *in situ* analyses. One such potential embodiment is the development of a micro-fabricated polymerase chain reaction (PCR) instrument. The amplification process from minute sample sizes and reaction volumes, and specific reaction sequence of the PCR technique plays favorably into the microdevice capabilities of on-going microfabrication technology. The development of this integrated micro-PCR system will lead to a highly automated, miniaturized, analytical instrument for very rapid *in situ* analyses and production of a variety of samples. The principles applied and problems solved in developing the PCR instrument will be used to further the application of microdevice-based instruments to other biochemical and chemical reactions and analyses.

Specific Claims

1) A microfabricated device(s) and/or series of devices for chemical and biochemical reaction manipulation, control, and detection. These micro devices may be exemplified by, but are not limited to, Lamb-wave devices. Other microfabricated devices that constitute the parts required to enhance or perform the reactions, manipulations, and treatments of specific chemical reactions and to provide for product detection are included herein.

2) The use of microfabricated devices to perform the polymerase chain reaction (PCR). The use of the inherent properties of such devices, for example, the Lamb-wave device to pump, mix, enhance, and detect the PCR reaction. The use electrokinetic effects to pump, separate, or preconcentrate PCR reactants and products on a microdevice. The use of integrated temperature-control devices to provide the temperature regime required by the PCR reaction. The use of microfabricated devices to provide the precise manipulation and the prevention of contamination of PCR products and reactants.

3) The use of microfabricated devices as indicated above to perform pre- and post-PCR reactions and manipulations, including but not limited to, cell lysing, secoundary-structure manipulation, reaction enhancement, sonochemistry, DNA hybridizations and detections, DNA vector manipulations, plasmid incorporations and other manipulations that lead-upto or utilize PCR products created on a microdevice as described.

4)The use of inherent properties of the microdevice to expedite, enhance, or otherwise manipulate and control such reactions. This includes but is not limited to: lysising of cells, reaction enhancement, sonochemistry, and manipulation of (e.g., unravelling) secondary, tertiary, or quaternary structure of reacting or product molecules. Other manipulations or reactants or products coupled with ultrasonication, or independently, such as but not limited to electrokinetic preconcentrating or pumping, surface modifications such as (DNA) hybridization or other reaction-controlling treatments .

5) Incorporation of the inherent sensing parameters or other sensing methodologies to monitor and provide feedback control of reactions in such microdevices. This includes, but is not limited to measurements of mass changes, density, viscosity with Lamb-wave devices, or with optical means such as with specialized optical fibers and/or detection windows of reactions and products. One example of many possible uses of such sensing parameters is to monitor the DNA denaturing and annealing in the DNA polymerase reaction via changes in the reaction solution viscosity. Optical monitoring of the PCR reactionwith DNA hybridized optical fibers is also just one more example of possible monitoring schemes.

6) Provision of temperature and motion control within the microdevice-based reactors and to therefore manipulate reaction conditions. This includes but is not limited to incorporation of heating elements on one, two, or several sides of an enclosed micro-reaction device such as indicated in Figure 2. This set-up, for example, can be use to provide the temperatures required in a biochemical reaction such as the DNA-polymerase reaction or in any other reaction requiring precise temperature control of small volumes.

7) The control of movement of reactants or products from isolated chambers into and out of such micro-reaction vessels and the use of similar device characteristics to mix reactants. Examples of such manipulations include, but are not limited to, Lamb-wave devices to pump reactants into (or products out of) and to mix reactants in micro-reaction vessels, or the use of electrokinetic effects between two ends of a chamber to pump or preconcentrate certain charged species of reactants or products.

Fig 2(a)

BIOCHEMICAL MICROFLOW CHAMBER
(TOP VIEW)

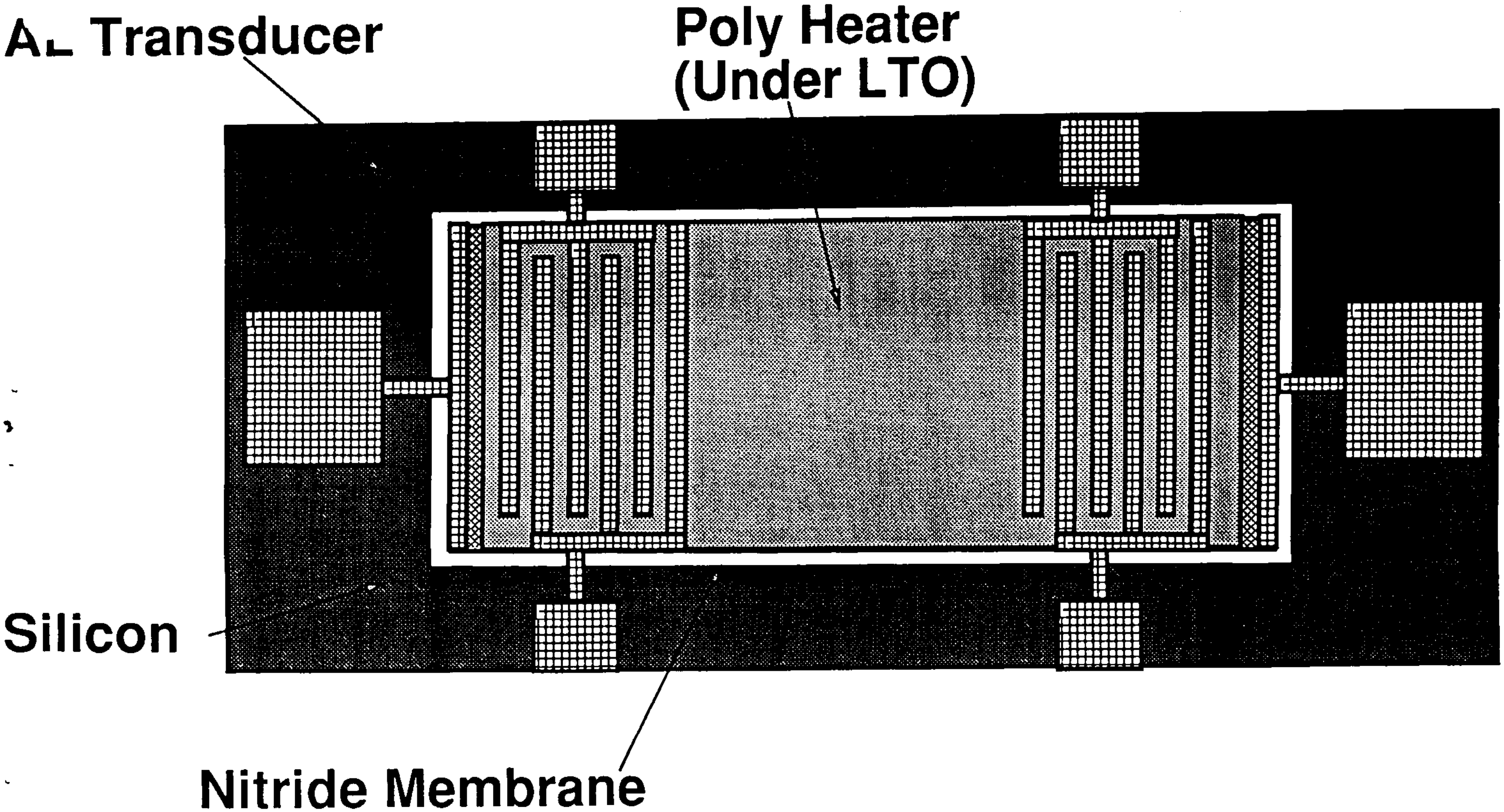


Fig 2 (b)

BIOCHEMICAL MICROFLOW CHAMBER